Acknowledgments

References

1. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53:5046–54. http://dx.doi.org/10.1128/AAC.00774-09
2. Peirano G, Lascols C, Hackel M, Hoban DJ, Pitout JD. Molecular epidemiology of *Enterobacteriaceae* that produce VIMs and IMPs from the SMART surveillance program. Diagn Microbiol Infect Dis. 2014;78:277–81. http://dx.doi.org/10.1016/j.diagmicrobio.2013.11.024
3. Peirano G, Bradford PA, Kazmierczak KM, Badal RE, Hackel M, Hoban DJ, et al. Global incidence of carbapenemase-producing *Escherichia coli* ST131. Emerg Infect Dis. 2014;20:1928–31. http://dx.doi.org/10.3201/eid2011.141388
4. Chou A, Roa M, Evangelista MA, Sulit AK, Lagamayo E, Torres BC, et al. Emergence of *Klebsiella pneumoniae* ST273 carrying bla_{NDM-7} and ST656 carrying bla_{NDM-1} in Manila, Philippines. Microb Drug Resist. 2016;22:585–8. http://dx.doi.org/10.1089/mdr.2015.0205
5. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: twenty-fifth informational supplement (M100–S25). Wayne (PA): The Institute, 2015.
6. Nordmann P, Poirel L, Dortet L. Rapid detection of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis. 2012;18:1503–7. http://dx.doi.org/10.3201/eid1809.120355
7. Milillo M, Kwak YI, Snesrud E, Waterman PE, Lesho E, McGann P. Rapid and simultaneous detection of bla_{KPC} and bla_{NDM} by use of multiplex real-time PCR. J Clin Microbiol. 2013;51:1247–9. http://dx.doi.org/10.1128/JCM.03316-12
8. Milillo M, Kwak YI, Snesrud E, Waterman PE, Lesho E, McGann P. Correction for Milillo et al., Rapid and simultaneous detection of blaKPC and blaNDM by use of multiplex real-time PCR. J Clin Microbiol. 2015;53:1460. http://dx.doi.org/10.1128/JCM.00373-15
9. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of carbapenem-resistant *Klebsiella pneumoniae* acquisition among hospitalized adults and effect of acquisition on mortality. Antimicrob Agents Chemother. 2008;52:1028–33. http://dx.doi.org/10.1128/AAC.01020-07
10. Kulkova N, Babalova M, Sokolova J, Krcmery V. First report of New Delhi metallo-β-lactamase-1–producing strains in Slovakia. Microb Drug Resist. 2015;21:117–20. http://dx.doi.org/10.1089/mdr.2013.0162

Address for correspondence: John Mark Velasco, Department of Virology, Armed Forces Research Institute of Medical Sciences, 315/6 Rajvithi Rd, Bangkok 10400, Thailand; email: John.Velasco.ca@afrims.org

Chronic Wasting Disease Prion Strain Emergence and Host Range Expansion

Allen Herbst,[1] Camilo Duque Velásquez,[1] Elizabeth Triscott, Judd M. Aiken, Debbie McKenzie

Author affiliation: University of Alberta, Edmonton, Alberta, Canada

Human and mouse prion proteins share a structural motif that regulates resistance to common chronic wasting disease (CWD) prion strains. Successful transmission of an emergent strain of CWD prion, H95+, into mice resulted in infection. Thus, emergent CWD prion strains may have higher zoonotic potential than common strains.

Chronic wasting disease (CWD) is a contagious prion disease of cervids that is spreading globally. CWD is enzootic in multiple cervid species, including deer and elk; the major foci of disease are Colorado/Wyoming (USA), Wisconsin/Illinois (USA), and Alberta/Saskatchewan (Canada). CWD is also present in captive cervids in South Korea and wild reindeer and moose in Norway (https://www.nwhc.usgs.gov/images/cwd/cwd_map.jpg). CWD results from the conformational transformation of the host-encoded cellular prion protein (PrP^C) into protease-resistant, detergent-insoluble, β-sheet rich, amyloidogenic conformers, termed prions (PrP^{CWD}). Within their conformation, prion strains encipher the information that directs the templated misfolding and aggregation of PrP^C molecules into additional prions (*1*).

Although the sequence homology of PrP among mammals is high, the ability of particular prion strains to cause disease in different species is determined by the conformational compatibility between a given strain and the host PrP^C (*2*). We

[1]These authors contributed equally to this article.

previously identified 2 strains of CWD prion in white-tailed deer (*3*), Wisc-1 and H95+; these strains exhibit distinct biological properties in deer and transgenic cervidized mice. To ascertain the host range of different strains from cervids, we inoculated CWD prions isolated from experimentally infected deer with different *PRNP* genotypes (Q95G96 [wild type (wt)], S96/wt, H95/wt, and H95/S96) and from elk (CWD2 strain) into hamsters and mice. All isolates have been successfully transmitted into transgenic mice expressing wt cervid PrP and contain high titers of CWD prions (*3*).

Mice inoculated with H95+ CWD prions succumbed to clinical disease at 575 ± 47 or 692 ± 9 days, depending on the H95+ isolate (Table). Mice inoculated with Wisc-1 or elk CWD or uninfected deer homogenates were euthanized at day 708 after infection with no signs of prion disease. Clinical signs of H95+ CWD in C57Bl/6 mice included ataxia, lethargy, tail rigidity, and dermatitis. Protease-resistant PrP^CWD was present in all mice infected with H95+ prions and was not detected in mice infected with Wisc-1 or CWD2 (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/9/16-1474-Techapp1.pdf).

In contrast to mice, hamsters succumbed to clinical disease when inoculated with Wisc-1 CWD prions but were less susceptible to H95+ CWD prions (Table). Clinical signs of CWD in hamsters began with lethargy and, upon arousal, retrocollis; as the disease progressed, lethargy declined with increased dystonic movement including ataxia and tremors. Hyperesthesia was not observed. Subclinical disease (no clinical signs but PrP-res positive by Western blot) was observed in a subset of hamsters (online Technical Appendix).

Successful interspecies prion transmission at the molecular level depends on the compatibility of the invading prion conformers and structural determinants imposed by host PrP^C. One structural motif is the loop region between β sheet 2 and α helix 2 of PRP^C at aa 170–174 (online Technical Appendix). Host species containing PrP^C molecules with a flexible β2-α2 loop (mice and humans) are hypothesized to be incompatible with prions derived from species containing a rigid loop (deer and elk) (*4,5*). Previous attempts to transmit CWD to mice have failed (*6,7*). Our data show that prions from a prototypic rigid-loop species (deer) can transmit to a flexible-loop species (mice). The transmission is strain dependent. H95+ overrides the conformational restriction imposed by the mouse PrP flexible loop that Wisc-1 and CWD2 cannot overcome, suggesting that the invading prion strain is a dominant contributor to the species/transmission barrier. How the N terminal amino acid polymorphism (Q95H) affects the conformation of PrP, altering the deer-to-mouse transmission barrier, is unknown. Further structural studies may clarify the effect of N terminal residues on β2-α2 loop rigidity.

Transmission of H95+ CWD prions to mice further confirms the value of specifying strain when defining species barriers. Experimental transmission of CWD prion into macaques and transgenic mice expressing human PrP suggests a considerable transmission barrier to CWD prions (although squirrel monkeys are susceptible), and human prion protein is converted inefficiently in vitro (*8,9*). Successful infection of a flexible-loop species (mice) with H95+ CWD raises concerns for the potential pathogenicity of H95+ prions to other flexible-loop species. Transmission studies with Wisc-1 and H95+ in transgenic humanized and bovinized mice are ongoing.

The increasing prevalence of CWD indicates selection for cervids with resistance alleles, such as S96 and H95. Genetic resistance to a given prion strain selects for the emergence of novel prion strains with altered properties such as H95+ and Nor98 (*3,10*). The iterative transmission of CWD prions to cervids with protective alleles of PrP^C and the consequent emergence of new CWD prion strains highlights the dynamics of the CWD panzootic and the value of characterizing the host range of emergent CWD prion strains.

Table. Results of CWD prion inoculation into rodents*

Recipient and CWD inocula	No.	PrP-res+		Incubation period, d
		Clinical	Subclinical	
Mice				
wt/wt	6	0	0	NA
S96/wt	6	0	0	NA
H95/wt	7	5	2	669, 671, 706, 706, 706
H95/S96	7	7	0	306, 593, 593, 593, 593, 673, 675
Elk	4	0	0	NA
Control	4	0	0	NA
Hamsters				
wt/wt	8	3	5	652, 653, 653
S96/wt	8	1	4	634
H95/wt	8	1	6	652
H95/S96	8	0	1	NA
Elk	8	2	2	673, 719
Control	8	0	0	NA

*Mice infected with CWD prions were observed for up to 708 d; hamsters infected with white-tailed deer and elk CWD prions were observed for 659 and 726 d, respectively. Control mice and hamsters were inoculated with brain homogenates from CWD-negative wt/wt deer. CWD, chronic wasting disease; NA, not applicable; PrP-res+, positive for proteinase-K–resistant prion protein; wt, wild type.

References

1. Bessen RA, Marsh RF. Identification of two biologically distinct strains of transmissible mink encephalopathy in hamsters. J Gen Virol. 1992;73:329–34. http://dx.doi.org/10.1099/0022-1317-73-2-329
2. Capobianco R, Casalone C, Suardi S, Mangieri M, Miccolo C, Limido L, et al. Conversion of the BASE prion strain into the BSE strain: the origin of BSE? PLoS Pathog. 2007;3:e31. http://dx.doi.org/10.1371/journal.ppat.0030031
3. Duque Velásquez C, Kim C, Herbst A, Daude N, Garza MC, Wille H, et al. Deer prion proteins modulate the emergence and adaptation of chronic wasting disease strains. J Virol. 2015; 89:12362–73. http://dx.doi.org/10.1128/JVI.02010-15
4. Gossert AD, Bonjour S, Lysek DA, Fiorito F, Wüthrich K. Prion protein NMR structures of elk and of mouse/elk hybrids. Proc Natl Acad Sci U S A. 2005;102:646–50. http://dx.doi.org/10.1073/pnas.0409008102
5. Sigurdson CJ, Nilsson KP, Hornemann S, Heikenwalder M, Manco G, Schwarz P, et al. De novo generation of a transmissible spongiform encephalopathy by mouse transgenesis. Proc Natl Acad Sci U S A. 2009;106:304–9. http://dx.doi.org/10.1073/pnas.0810680105
6. Raymond GJ, Raymond LD, Meade-White KD, Hughson AG, Favara C, Gardner D, et al. Transmission and adaptation of chronic wasting disease to hamsters and transgenic mice: evidence for strains. J Virol. 2007;81:4305–14. http://dx.doi.org/10.1128/JVI.02474-06
7. Kurt TD, Bett C, Fernández-Borges N, Joshi-Barr S, Hornemann S, Rülicke T, et al. Prion transmission prevented by modifying the β2-α2 loop structure of host PrP^C. J Neurosci. 2014;34:1022–7. http://dx.doi.org/10.1523/JNEUROSCI.4636-13.2014
8. Race B, Meade-White KD, Phillips K, Striebel J, Race R, Chesebro B. Chronic wasting disease agents in nonhuman primates. Emerg Infect Dis. 2014;20:833–7. http://dx.doi.org/10.3201/eid2005.130778
9. Barria MA, Telling GC, Gambetti P, Mastrianni JA, Soto C. Generation of a new form of human PrP(Sc) in vitro by interspecies transmission from cervid prions. J Biol Chem. 2011;286:7490–5. http://dx.doi.org/10.1074/jbc.M110.198465
10. Le Dur A, Béringue V, Andréoletti O, Reine F, Laï TL, Baron T, et al. A newly identified type of scrapie agent can naturally infect sheep with resistant PrP genotypes. Proc Natl Acad Sci U S A. 2005;102:16031–6. http://dx.doi.org/10.1073/pnas.0502296102

Address for correspondence: Debbie McKenzie, University of Alberta, 120 Brain and Aging Research Building, Edmonton, AB T6G 2R3, Canada; email: debbie.mckenzie@ualberta.ca

Rabies Virus Transmission in Solid Organ Transplantation, China, 2015–2016

Shuilian Chen,[1] Heng Zhang,[1] Meiling Luo, Jingfang Chen, Dong Yao, Faming Chen,[2] Ruchun Liu,[2] Tianmu Chen[2]

Author affiliation: Changsha Center for Disease Control and Prevention, Changsha, Hunan, China

We report rabies virus transmission among solid organ transplantation recipients in Changsha, China, in 2016. Two recipients were confirmed to have rabies and died. Our findings suggest that more attention should be paid to the possibility of rabies virus transmission through organ transplantation for clinical and public health reasons.

In 2016, Zhou et al. reported a case of rabies virus transmission in China that was probably a result of organ transplantation (1). We report on rabies transmission that occurred among solid organ transplant recipients in Changsha, China, during December 2015–January 2016.

In November 2015, the donor, a previously healthy boy, showed development of fever, insomnia, and agitation. On day 6 of infection, these symptoms progressed, and he was sent to a healthcare center. At this time, he experienced weariness, no desire to drink water, poor appetite, and panic. One day later, he began vomiting, and was admitted to a local hospital (hospital A), where he exhibited anemophobia, convulsions, limb rigidity, and hypersalivation. The patient was moved to hospital B (days 7–14) in Changsha. At admission, some examination findings indicated a possibility of viral encephalitis (online Technical Appendix, Table 1, https://wwwnc.cdc.gov/EID/article/23/9/16-1704-Techapp1.pdf). Subhypothermia hibernation therapy and assisted ventilation were administered within 72 hours of admission, and the patient's vital signs became stable. On day 10, hyponatremia was observed, and on day 11, the patient again became febrile and tachycardic, with hypertensive abdominal distention and alimentary tract hemorrhage. On day 13, viral encephalitis was diagnosed, and rabies was suspected. However, rabies virus antibody tests performed on serum samples by using ELISA yielded negative results.

On day 14, the patient was transferred to hospital C, where he became comatose and was declared brain dead. Permission was granted for organ donation, because no specific pathogen had been detected and China's organ

[1]These authors contributed equally to this article.

[2]These authors contributed equally to this article.

Chronic Wasting Disease Prion Strain Emergence and Host Range Expansion

Technical Appendix

Additional Data

Brain homogenates from all mice (experimentally infected animals and uninfected controls) were analyzed for the presence of PrP-res. Proteinase K-resistant PrP was detected in all mice infected with H95$^+$ prions (Figure 1) demonstrating that H95$^+$ is 100% penetrant. PrP-res was not, however, detected in mice infected with the Wisc-1 strain from wt/wt or S96/wt deer or CWD2 from elk.

A similar analysis of brain homogenates from hamsters was also performed. Wisc-1 CWD was preferentially transmitted to hamsters upon primary passage (Figure 2). Wt/wt CWD caused clinical disease or subclinical accumulation of PrP-res in exposed hamsters. Transmission of S96/wt and H95/wt CWD prions resulted, primarily, in subclinical disease. Inoculation of elk CWD prions resulted in clinical disease in two out of five hamsters and subclinical disease in one animal. The H95/S96 isolate transmitted inefficiently with only one of eight hamsters having subclinical disease. This subclinical infection may be due to Wisc-1 in the H95/S96 deer isolate. Hamsters inoculated with uninfected deer brain homogenate did not show signs of prion disease nor accumulate PrP-res.

Secondary structure of host PrP influences the ability of a strain to propagate in a new host. Based on NMR studies, it has been hypothesized that amino acid variation at residues 170-175 (Figure 3) in PrP^C results in a loop structure between beta-sheet 2 and alpha-helix 2 (β2-α2) whose flexibility influences the outcome of interspecies transmissions (*1–7*). Mice and humans share a flexible loop sequence (SNQNN) whereas deer and elk possess a rigid loop (NNQNT). Multiple attempts to transmit CWD prions to mice have failed (*5–11*). The transmission of prions occurs more readily when the loop structures match, e.g. BSE transmits to humans and mice (*12,13*). Experimental alteration of the mouse flexible loop to a rigid loop facilitated CWD

transmission (*4,7*). Our data suggests emergent CWD strain H95$^+$ can overcome the conformational restriction imposed by the loop.

Methods and Materials

This study was conducted in accordance with the guidelines of the Canadian Council on Animal Care. The protocols used were approved by the Institutional Animal Care and Use Committees at the University of Alberta.

Brain homogenates were prepared from clinically-affected white-tailed deer (*Odocoileus virginianus*) of defined genotypes (*14,15*) or as a pool from three captive CWD-positive 132M/M elk (*Cervus canadensis*) (*16,17*), a kind gift from Catherine Graham. Four different white-tailed deer CWD isolates were used, one isolate was derived from a deer homozygous for the most common *PRNP* gene (wt = Q95G96) and three heterozygous deer (H95/wt, 96S/wt and H95/S96) expressing *PRNP* allele variants. These isolates have been extensively characterized in transgenic mice (*15*). Weanling C57Bl/6 mice were intracerebrally inoculated with 30 µl of 1% brain homogenate. Weanling Syrian Golden hamsters (*Mesocricetus auratus*) were intracerebrally inoculated with 50 µl of 10% brain homogenates (elk CWD was passaged at 1% brain homogenate). Mock-infected controls received brain homogenate from unaffected, PrP-res negative deer. Mice and hamsters were monitored for onset of clinical signs and disease progression. Individual incubation periods are expressed as the number of days post-inoculation (dpi) and were calculated from the time that the mice were inoculated until the time that clinical disease was established. Experiments were terminated at 708 dpi (mice) and 659 dpi (hamsters); all remaining animals were euthanized and assayed for PrP-res.

Brains were collected from mice and hamsters, flash frozen and stored at -80 until analysis. Tissues were homogenized to 10% (wt/vol) in sterile water with a tissue disruptor (Omniprep) and disposable homogenization tubes and beads. PrP-res was detected following proteinase K digestion and immunoblotting as previously described (*15*). Blots were probed with antibodies SAF83 (mice; Cayman Chemical, Michigan) or 3F4 (hamsters; a kind gift from Richard Rubenstein) monoclonal antibodies.

References

1. Gossert AD, Bonjour S, Lysek DA, Fiorito F, Wüthrich K. Prion protein NMR structures of elk and of mouse/elk hybrids. Proc Natl Acad Sci U S A. 2005;102:646–50. PubMed http://dx.doi.org/10.1073/pnas.0409008102

2. Gorfe AA, Caflisch A. Ser170 controls the conformational multiplicity of the loop 166-175 in prion proteins: implication for conversion and species barrier. FASEB J. 2007;21:3279–87. PubMed http://dx.doi.org/10.1096/fj.07-8292com

3. Sigurdson CJ, Nilsson KP, Hornemann S, Heikenwalder M, Manco G, Schwarz P, et al. De novo generation of a transmissible spongiform encephalopathy by mouse transgenesis. Proc Natl Acad Sci U S A. 2009;106:304–9. PubMed http://dx.doi.org/10.1073/pnas.0810680105

4. Sigurdson CJ, Nilsson KP, Hornemann S, Manco G, Fernández-Borges N, Schwarz P, et al. A molecular switch controls interspecies prion disease transmission in mice. J Clin Invest. 2010;120:2590–9. PubMed http://dx.doi.org/10.1172/JCI42051

5. Bett C, Fernández-Borges N, Kurt TD, Lucero M, Nilsson KP, Castilla J, et al. Structure of the β2-α2 loop and interspecies prion transmission. FASEB J. 2012;26:2868–76. PubMed http://dx.doi.org/10.1096/fj.11-200923

6. Kurt TD, Bett C, Fernández-Borges N, Joshi-Barr S, Hornemann S, Rülicke T, et al. Prion transmission prevented by modifying the β2-α2 loop structure of host PrPC. J Neurosci. 2014;34:1022–7. PubMed http://dx.doi.org/10.1523/JNEUROSCI.4636-13.2014

7. Kurt TD, Jiang L, Fernández-Borges N, Bett C, Liu J, Yang T, et al. Human prion protein sequence elements impede cross-species chronic wasting disease transmission. J Clin Invest. 2015;125:1485–96. PubMed http://dx.doi.org/10.1172/JCI79408

8. Browning SR, Mason GL, Seward T, Green M, Eliason GA, Mathiason C, et al. Transmission of prions from mule deer and elk with chronic wasting disease to transgenic mice expressing cervid PrP. J Virol. 2004;78:13345–50. PubMed http://dx.doi.org/10.1128/JVI.78.23.13345-13350.2004

9. Raymond GJ, Raymond LD, Meade-White KD, Hughson AG, Favara C, Gardner D, et al. Transmission and adaptation of chronic wasting disease to hamsters and transgenic mice: evidence for strains. J Virol. 2007;81:4305–14. PubMed http://dx.doi.org/10.1128/JVI.02474-06

10. Meyerett C, Michel B, Pulford B, Spraker TR, Nichols TA, Johnson T, et al. In vitro strain adaptation of CWD prions by serial protein misfolding cyclic amplification. Virology. 2008;382:267–76. PubMed http://dx.doi.org/10.1016/j.virol.2008.09.023

11. Trifilo MJ, Ying G, Teng C, Oldstone MB. Chronic wasting disease of deer and elk in transgenic mice: oral transmission and pathobiology. Virology. 2007;365:136–43. PubMed http://dx.doi.org/10.1016/j.virol.2007.03.032

12. Bruce ME, Will RG, Ironside JW, McConnell I, Drummond D, Suttie A, et al. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature. 1997;389:498–501. PubMed http://dx.doi.org/10.1038/39057

13. Hill AF, Desbruslais M, Joiner S, Sidle KC, Gowland I, Collinge J, et al. The same prion strain causes vCJD and BSE. Nature. 1997;389:448–50, 526. PubMed http://dx.doi.org/10.1038/38925

14. Johnson CJ, Herbst A, Duque-Velasquez C, Vanderloo JP, Bochsler P, Chappell R, et al. Prion protein polymorphisms affect chronic wasting disease progression. PLoS One. 2011;6:e17450. PubMed http://dx.doi.org/10.1371/journal.pone.0017450

15. Duque Velásquez C, Kim C, Herbst A, Daude N, Garza MC, Wille H, et al. Deer Prion Proteins Modulate the Emergence and Adaptation of Chronic Wasting Disease Strains. J Virol. 2015;89:12362–73. PubMed http://dx.doi.org/10.1128/JVI.02010-15

16. Pushie MJ, Shaykhutdinov R, Nazyrova A, Graham C, Vogel HJ. An NMR metabolomics study of elk inoculated with chronic wasting disease. J Toxicol Environ Health A. 2011;74:1476–92. PubMed http://dx.doi.org/10.1080/15287394.2011.618977

17. Angers RC, Kang HE, Napier D, Browning S, Seward T, Mathiason C, et al. Prion strain mutation determined by prion protein conformational compatibility and primary structure. Science. 2010;328:1154–8. PubMed http://dx.doi.org/10.1126/science.1187107
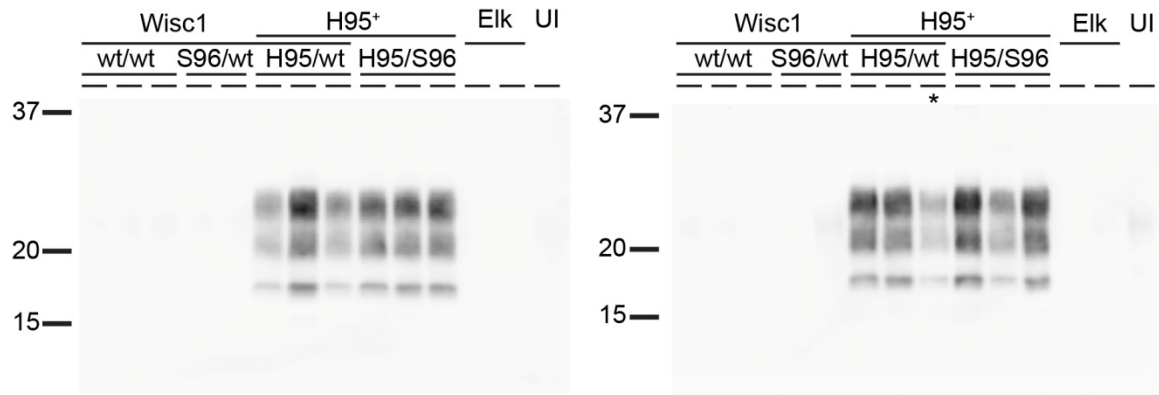
Figure 1. Representative western blot analysis of PrP-res on primary passage of CWD in mice. Each lane is an individual mouse brain homogenate sample treated with 50 µg/ml of proteinase K; equivalent volumes were loaded in each well. Only mice infected with H95+ isolates accumulated PK-resistant PrP. UI is a brain homogenate from a mouse inoculated with CWD negative wt/wt deer brain homogenate. Blots were probed with monoclonal antibody SAF83 at a 1:10,000 dilution. An asterisk denotes a subclinical mouse.
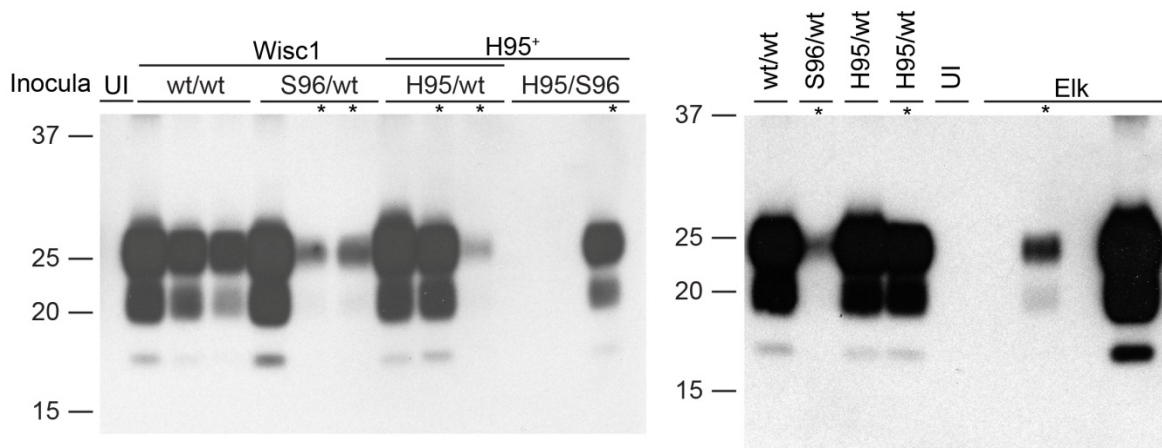

Figure 2. Representative western blot analysis of PrP-res on primary passage of CWD prions into Syrian golden hamsters. A. Brain homogenates from each passage line (10 µl of 10% brain homogenate) were treated with 100 µg/ml of proteinase K and 10 µl of each digested sample was loaded. One of 8 hamsters inoculated with H95/S96 CWD accumulated PK-resistant PrP. UI is a brain homogenate from a mock-infected hamster. Blots were probed with monoclonal antibody 3F4 at a 1:10,000 dilution. Asterisks denote subclinically affected hamsters.

```
              10        20        30        40        50        60
--MANLGCWMLVLFVATWSDLGLCKKRPKPGG-WNTGGSRYPGQGSPGGNRYPPQGGGGWGQPHG Homo sapiens
--MANLGYWLLALFVTMWTDVGLCKKRPKPGG-WNTGGSRYPGQGSPGGNRYPPQGG-TWGQPHG Mus musculus
MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGGWGQPHG Odocoileus virginianus wt
MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGGWGQPHG Odocoileus virginianus H95
MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGGWGQPHG Odocoileus virginianus S96
MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPPQGGGGWGQPHG Cervus canadensis
--MANLSYWLLALFVAMWTDVGLCKKRPKPGG-WNTGGSRYPGQGSPGGNRYPPQGGGTWGQPHG Mesocricetus auratus


              70        80        90       100       110       120
GGWGQPHGGGWGQPHGGGWGQPHGGG-WGQGGGTHSQWNKPSKPKTNMKHMAGAAAAGAVVGGLG Homo sapiens
GGWGQPHGGSWGQPHGGSWGQPHGGG-WGQGGGTHNQWNKPSKPKTNLKHVAGAAAAGAVVGGLG Mus musculus
GGWGQPHGGGWGQPHGGGWGQPHGGGGWG[QG]G-THSQWNKPSKPKTNMKHVAGAAAAGAVVGGLG Odocoileus virginianus wt
GGWGQPHGGGWGQPHGGGWGQPHGGGGWG[HG]G-THSQWNKPSKPKTNMKHVAGAAAAGAVVGGLG Odocoileus virginianus H95
GGWGQPHGGGWGQPHGGGWGQPHGGGGWG[QS]G-THSQWNKPSKPKTNMKHVAGAAAAGAVVGGLG Odocoileus virginianus S96
GGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGG-THSQWNKPSKPKTNMKHVAGAAAAGAVVGGLG Cervus canadensis
GGWGQPHGGGWGQPHGGGWGQPHGGG-WGQGGGTHNQWNKPSKPKTNMKHMAGAAAAGAVVGGLG Mesocricetus auratus


        130       140       150       160       170       180       190
GYMLGSAMSRPIIHFGSDYEDRYYRENMHRYPNQVYYRPMDEY[SNQN]NFVHDCVNITIKQHTVTT Homo sapiens
GYMLGSAMSRPMIHFGNDWEDRYYRENMYRYPNQVYYRPVDQY[SNQN]NFVHDCVNITIKQHTVTT Mus musculus
GYMLGSAMSRPLIHFGNDYEDRYYRENMYRYPNQVYYRPVDQY[NNQN]TFVHDCVNITVKQHTVTT Odocoileus virginianus wt
GYMLGSAMSRPLIHFGNDYEDRYYRENMYRYPNQVYYRPVDQY[NNQN]TFVHDCVNITVKQHTVTT Odocoileus virginianus 95H
GYMLGSAMSRPLIHFGNDYEDRYYRENMYRYPNQVYYRPVDQY[NNQN]TFVHDCVNITVKQHTVTT Odocoileus virginianus 96S
GYMLGSAMSRPLIHFGNDYEDRYYRENMYRYPNQVYYRPVDQY[NNQN]TFVHDCVNITVKQHTVTT Cervus canadensis
GYMLGSAMSRPMMHFGNDWEDRYYRENMNRYPNQVYYRPVDQY[NNQN]NFVHDCVNITIKQHTVTT Mesocricetus auratus


          200       210       220       230       240       250
TTKGENFTETDVKMMERVVEQMCITQYERESQAYYQ--RGSSMVLFSSPPVILLISFLIFLIVG Homo sapiens
TTKGENFTETDVKMMERVVEQMCVTQYQKESQAYYDGRRSSSTVLFSSPPVILLISFLIFLIVG Mus musculus
TTKGENFTETDIKMMERVVEQMCITQYQRESQAYYQ--RGASVILFSSPPVILLISFLIFLIVG Odocoileus virginianus wt
TTKGENFTETDIKMMERVVEQMCITQYQRESQAYYQ--RGASVILFSSPPVILLISFLIFLIVG Odocoileus virginianus H95
TTKGENFTETDIKMMERVVEQMCITQYQRESQAYYQ--RGASVILFSSPPVILLISFLIFLIVG Odocoileus virginianus S96
TTKGENFTETDIKMMERVVEQMCITQYQRESEAYYQ--RGASVILFSSPPVILLISFLIFLIVG Cervus canadensis
TTKGENFTETDIKIMERVVEQMCTTQYQKESQAYYDGRRSS-AVLFSSPPVILLISFLIFLMVG Mesocricetus auratus
```

Figure 3. Alignment of PrP amino acid sequences from humans, cervids and rodents. Numbering is based on the human sequence. Deer polymorphisms at 95, 96 (human residues 91, 92 respectively) and residues forming the β2-α2 loop are boxed.